Fluorescence Dilution Technique for Measurement of Cardiac Output and Circulating Blood Volume in Healthy Human Subjects

Jean-Michel I. Maarek, Dr.Eng.,* Daniel P. Holschneider, M.D.,† Eduardo H. Rubinstein, M.D., Ph.D.‡

Background: The authors previously validated in an animal model a new indicator dilution technique for measuring cardiac output and circulating blood volume by recording transcutaneously the fluorescence of circulating indocyanine green with an optical probe placed on the skin surface. The current study compared fluorescence dilution recordings recorded from several locations on the human face in terms of signal intensity and stability and estimated the subjects' cardiac output and circulating blood volume from the recordings.

Methods: Fluorescence dilution traces were recorded transcutaneously in six healthy human volunteers after rapid intravenous injection of 1 mg indocyanine green. Three placements of the optical probe were tested: the nose ala, the ear lobe, and the temple area. In three subjects, the recordings were calibrated in terms of circulating indocyanine green concentration to estimate cardiac output and circulating blood volume.

Results: Fluorescence dilution traces had the same duration for the three locations, but the recordings obtained from the nose ala and the ear lobe were twice as intense as those from the temple. The fluorescence intensity at each site was linearly related to the local laser Doppler perfusion index. The coefficient of variation for the area under the first pass curve (inversely proportional to cardiac output) was approximately 6% for triplicate measurements at the same location. Cardiac output and circulating blood volume derived from the fluorescence recordings were in the normal range.

Conclusions: The study demonstrates that intense and reproducible fluorescent dilution signals can be measured transcutaneously in healthy humans and could potentially be used to measure cardiac output and circulating blood volume minimally invasively.

CARDIAC output and circulating blood volume are important parameters in the clinical assessment of circulat...
dilution signal could be detected transcutaneously on the basis of fluorescence rather than absorption, as described above. Our recent experiments in rabbits suggest the feasibility of this approach, with a close correlation of blood volume determined by transcutaneous ICG fluorescence with blood volume obtained with the Evans blue dye dilution method.8 In principle, such a simplified method for tracking acute changes in blood volume could benefit several patient populations, including patients undergoing kidney dialysis in whom excessive blood volume shifts may result in transient hypotension.14

We describe the first trial of the ICG fluorescence dilution technique in healthy human subjects. The goals of this study were (1) to measure the ICG fluorescence from several sites on the body surface potentially favorable to optical sensing and probe placement and to compare the fluorescence in terms of signal intensity and stability, and (2) to calibrate the fluorescence signal intensity in terms of circulating ICG concentration and to determine whether the technique yields realistic estimates of cardiac output and circulating blood volume.

Materials and Methods

Subject Preparation and Instrumentation

Six healthy volunteers were enrolled for the study. The subjects were screened to exclude allergy to iodine and seafood products, history of disease of the cardiovascular or peripheral vascular system, diseases of the lung, liver, skin, or blood, and conditions that might affect peripheral perfusion (including diabetes mellitus), as well as obesity, and ongoing treatment with sympathomimetics or β-blockers. Also excluded were female volunteers with a positive urine pregnancy test result. All subjects gave written informed consent to participate in the experimental protocol approved by the University of Southern California Institutional Review Board (Los Angeles, California). Biometric data for the subjects are provided in table 1. The subjects did not fast before the study. They rested supine during the measurements. At the beginning of the study, two 2-in, 18-gauge intravenous catheters were placed in the right and left antecubital veins for injection of the ICG solution and for blood sampling, respectively. Each line was connected to a drip of heparinized 5% dextrose solution (approximately 30 ml/h, 1 U heparin/ml) through a three-way stopcock.

A customized optical probe (415-250; Perimed, Jarfalla, Sweden) was used to obtain paired measurements of the ICG fluorescence dilution intensity and the laser Doppler perfusion signal from the same location. The probe (3 mm diameter) contained three 125-μm-diameter glass optical fibers in its center for excitation of the ICG fluorescence and a ring of 10 fibers—also 125 μm in diameter—located 2 mm from the center bundle for detection of the fluorescence intensity. The excitation fibers were coupled to a 782-nm laser diode (SRT-F785S; Micro Laser Systems, Garden Grove, CA) whose output light intensity (approximately 5 mW) was modulated at 1 kHz. The fluorescence emission captured at the skin surface by the detection fibers was directed toward an 830-nm interferential filter (079-2230; OptoSigma, Santa Ana, CA) placed in front of a photomultiplier tube (H7732-10; Hamamatsu, Bridgewater, NJ). The photomultiplier output signal was demodulated with a lock-in amplifier (SR 830; Stanford Research Systems, Sunnyvale, CA), which also generated the 1-kHz modulation of the excitation light intensity at the level of the laser diode driver (CP 200; Micro Laser Systems). The wavelengths of excitation and detection were selected to maximize the intensity of the ICG fluorescence.9 The intensity of the laser emission and the gain of the detection system were kept constant throughout the study to allow for comparison of the detected fluorescence intensities across subjects.

Additional optical fibers in the probe (1 excitation fiber in the center and 10 detection fibers alternating at 2 mm from the center with the ICG detection fibers) were connected to a 780-nm laser Doppler perfusion monitor (PF 5010; Perimed) for measurement of local perfusion at the site from which the transcutaneous fluorescence dilution curve was recorded. Interference between the optical emissions of the two laser sources prevented simultaneous measurement of the ICG fluorescence and laser Doppler perfusion. Therefore, the laser Doppler perfusion signal was measured from the same location on the skin surface as the ICG fluorescence and within 5 min before and after each fluores-

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>HR, beats/min</th>
<th>Systemic Blood Pressure, Systolic/Diastolic, mmHg</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>164</td>
<td>57</td>
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<td>112/72</td>
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<td>2</td>
<td>24</td>
<td>M</td>
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<td>67</td>
<td>81</td>
<td>128/62</td>
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<tr>
<td>3</td>
<td>34</td>
<td>M</td>
<td>164</td>
<td>57</td>
<td>82</td>
<td>108/69</td>
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<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>155</td>
<td>48</td>
<td>74</td>
<td>107/65</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>F</td>
<td>178</td>
<td>72</td>
<td>64</td>
<td>109/54</td>
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<tr>
<td>6</td>
<td>26</td>
<td>M</td>
<td>173</td>
<td>82</td>
<td>67</td>
<td>138/70</td>
</tr>
</tbody>
</table>

Table 1. Demographics of the Subjects

Heart rate (HR) and systemic blood pressure were measured with the subject supine, just before the start of the tests. Subjects 1 and 3 were the same person studied twice with a 7-month interval between studies.

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cence measurement when the output of the ICG excitation laser diode was temporarily turned off.

A 1-cm-diameter heating ring connected to a thermostatic heater (PF 5020; Perimed) with feedback control maintained the local temperature at the site of measurement at 40°C to provoke stable vasodilation of the skin microvasculature while remaining below the nociceptive threshold. The optical probe and the heating ring were held in stable contact with the skin by means of an articulated arm connected to a modified semirigid surgical lamp headband. Three locations on the right side of the face were tested: the side of the nose at the level of the ala, the ear lobe, and the temple area. The nose ala and ear lobe locations were selected because successful optical absorption measurements of circulating ICG dye have been obtained from these sites with the pulse dye densitometry\(^5\),\(^12\) and ear densitometry\(^15\) techniques. The temple location was tested to assess whether an intense fluorescence signal could be detected from ICG dye circulating in the superficial temporal artery. The exact probe placement for the temple area was mapped by a handheld Doppler ultrasound pencil probe to an area overlying the superficial temporal artery, near the attachment of the external ear.

A four-channel analog–digital converter module (PowrErlab/4P; AD Instruments, Colorado Springs, CO) digitized (100 Hz), displayed, and stored the transcutaneous ICG fluorescence dilution traces and the laser Doppler perfusion readings continuously during the study. The transcutaneous fluorescence and laser Doppler measurements were extracted from this record for off-line analysis.

**Experimental Protocol**

The optical probe and heating ring were placed on the nose ala, and the heating unit was turned on 10 min before starting the ICG injections to allow for local perfusion to increase and stabilize, as indicated by the initial rise and subsequent plateau of the laser Doppler perfusion reading. For each ICG injection, the injection syringe was filled with 1.5 ml of 0.67 mg/ml ICG solution in 5% dextrose for the injection of 1 mg ICG through the catheter in the right antecubital vein. In pilot studies, we observed that injection of such a small volume resulted in a delayed and sluggish ICG fluorescence dilution curves, likely the result of inertia and fragmentation of the bolus in the veins of the arm.\(^16\) Such artifacts were avoided by rapidly injecting 5 ml of 5% dextrose solution after the ICG bolus. The subjects were encouraged to hold their breath for approximately 10 s starting just before the ICG injection and coinciding with exhalation to minimize fluctuations of the cardiac output associated with tidal breathing.\(^2\) A well-defined ICG fluorescence first pass trace followed by a recirculation hump was clearly detected for each ICG injection, with the initial rise of the fluorescence signal observed within 10–15 s after injection (fig. 1). Thereafter, the fluorescence intensity gradually decayed and returned to baseline in less than 10 min as the ICG was gradually eliminated from the circulation by the liver.\(^9\),\(^17\) Three ICG injections were performed 10 min apart, after which the probe was moved successively to the other two locations (ear lobe, then temple), with repetition of the measurement sequence for each location.

Calibration of the transcutaneous fluorescence intensity in terms of circulating ICG concentration was performed in three subjects when the optical probe was on the nose ala and on the ear lobe. For each site, after completion of the three ICG injections, a larger dose of ICG (15–25 mg) was rapidly injected. The fluorescence was recorded for 15 min while the ICG mixed homogeneously with the circulating blood volume and was progressively removed from the circulation. Within 3 min of the ICG injection, when the dye had completely mixed in the circulating blood volume,\(^14\) the fluorescence intensity had decreased to near the peak fluorescence signal intensity observed after the 1-mg injection dose. Four 3-ml blood samples were then consecutively obtained from the left venous catheter over an 8-min period, while the time-averaged fluorescence signal during each blood withdrawal was measured. The blood samples were kept in capped heparinized syringes on ice until the clinical experiment was completed. Thereafter,

![Image](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931065/ on 07/14/2018)
the blood samples were centrifuged (3,000 revolutions/min for 5 min), and the plasma ICG concentration was measured spectrophotometrically (DU 500 UV-Vis spectrophotometer; Beckman Coulter, Fullerton, CA). A calibration standard curve was constructed for each subject from an additional blood sample obtained at the end of the study by adding 4-μl aliquots of a 0.03-mg/ml ICG solution to 1.2 ml plasma to cover a plasma concentration range of 0–2.6 μg/ml. For each sample and the calibration standards, the fluorescence intensity was recorded at wavelengths between 750 and 850 nm that overlapped the absorption spectrum of ICG in plasma.14 The blood hematocrit was measured in duplicate from the blood sample used to derive the standard curve to calculate the ICG concentration in circulating blood from the ICG plasma concentrations.

**Data Analysis**

**Characterization of Fluorescence Traces.** First pass ICG fluorescence dilution traces were derived from the experimental recordings of ICG fluorescence by eliminating the recirculation artifact with an exponential fit of the trailing segment of the recording.2 The first pass ICG fluorescence dilution curves were characterized by several parameters (fig. 1) that defined the magnitude of the detected fluorescence intensity and the duration of the first pass peak, including the area under the first pass curve, the peak fluorescence intensity, the full-width at half-maximum, the circulation time, and the mean transit time. Reproducibility of these parameters defined the reproducibility of the fluorescence recordings for repeated trials on the same measurement site and between measurement sites.

**Calibration of Fluorescence Traces in Terms of Blood ICG Concentration.** The standard curve that related optical absorption to ICG concentration in plasma samples was established for each subject by multiple linear regression analysis using the spectrophotometric absorption readings at 750, 780, 805, and 850 nm as independent variables.18 The regression equation was then used to determine the ICG concentrations in the blood samples obtained during the calibration phase of the clinical study. Blood ICG concentration was estimated from the ICG plasma concentration and the central blood hematocrit without correction for the difference between central blood hematocrit and body hematocrit14:

\[
[\text{ICG}]_{\text{blood}} = (1 - \text{hematocrit}) \cdot [\text{ICG}]_{\text{plasma}}
\]

The four transcutaneous fluorescence readings measured at the times of blood sampling were then analyzed by linear regression to derive a scaling factor (slope of the regression line) to convert the experimental transcutaneous fluorescence in terms of circulating blood ICG concentration.

**Computation of Cardiac Output and Circulating Blood Volume.** Cardiac output was estimated in triplicate from the recordings obtained on the nose ala and the ear lobe by dividing the injected ICG dose (1 mg) by the area under the calibrated first pass fluorescence dilution trace.7 For each measurement site, an estimate of the circulating blood volume was derived using the recording used to calibrate the fluorescence traces in terms of blood ICG concentration, when the injected amount of ICG was 15–25 mg. An exponential fit of the fluorescence recording obtained from 3 to 10 min after the ICG injection (i.e., the declining plateau phase) was back-extrapolated to the instant at which the fluorescence signal diverged from the baseline to estimate the theoretical concentration ([ICG]₀) that would be obtained if the injected dye had homogeneously mixed in the entire circulating intravascular volume. Circulating blood volume BVICG was calculated as the ratio of the mass of the injected ICG divided by [ICG]₀.12 The time constant of the exponential fit (τICG) estimated the half-life of ICG metabolic disposal.

**Statistical Analysis**

The effect of the site of measurement on the parameters describing the fluorescence recordings was analyzed with a general linear model in which the anatomical location of the measurement was used as a between-subjects factor, and the three measurements at each location were used as a repeated-measures within-subjects factor. Post hoc comparison between individual locations was performed with a t test with Bonferroni correction. Linear regression analysis was used to relate the area under the fluorescence dilution traces and the peak fluorescence intensity to the laser Doppler perfusion. Statistical significance was set at \( P < 0.05 \).

**Results**

The area under the curve and the maximum fluorescence intensity measured at the nose ala and at the ear lobe were of equivalent magnitude and approximately twice as large as the corresponding measurements obtained from the temple area (table 2). In contrast, the full-width at half-maximum, the circulation time, and the mean transit time were not different for the three sites of measurement. This observation suggests that the same ICG dilution signal was measured from all three anatomical sites in terms of temporal dynamics and duration. The yield was higher when the optical probe was on the nose ala and on the ear lobe because the volume interrogated by the probe contained more circulating ICG and/or because less optical attenuation was present between the microvasculature and the external probe. None of the descriptors of the ICG fluorescence traces showed a significant trend on the repeated measure-
Table 2. Descriptors of Fluorescence Dilution Curves

<table>
<thead>
<tr>
<th></th>
<th>Nose Ala</th>
<th>Ear Lobe</th>
<th>Temple</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, V·s</td>
<td>12.7 ± 2.7</td>
<td>11.6 ± 3.4</td>
<td>6.7 ± 1.5</td>
<td>*</td>
</tr>
<tr>
<td>Max. intensity, V</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>*</td>
</tr>
<tr>
<td>FWHM, s</td>
<td>9.3 ± 1.7</td>
<td>9.5 ± 1.0</td>
<td>10.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Circ. time, s</td>
<td>19.3 ± 3.7</td>
<td>20.4 ± 3.6</td>
<td>20.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Mean transit time, s</td>
<td>10.9 ± 1.9</td>
<td>11.6 ± 1.5</td>
<td>12.8 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

Parameters used to describe the magnitude and duration of the first pass fluorescence dilution curves measured on the nose ala, the ear lobe, and the temple (mean ± SD; n = 18 [triplicate measurements of fluorescence traces on six subjects] for nose ala and ear lobe measurements; n = 9 [triplicate measurements of fluorescence traces on three subjects] for temple measurements). The mean transit time of the corrected fluorescence curve was computed as indicated in the legend of figure 1.

* Significant difference (P < 0.05) between locations identified using a general linear model using anatomical location as a between-subjects factor and the three measurements at each location as a repeated-measures within-subjects factor.

Table 3. Cardiac Output and Blood Volume Estimates

<table>
<thead>
<tr>
<th></th>
<th>CO, l/min</th>
<th>CI, l/min/m²</th>
<th>BV, l</th>
<th>BVI, ml/kg</th>
<th>TICG, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 4</td>
<td>5.3 ± 0.6</td>
<td>3.7 ± 0.4</td>
<td>3.9 ± 0.6</td>
<td>83 ± 13</td>
<td>238 ± 68</td>
</tr>
<tr>
<td>Subject 5</td>
<td>4.9 ± 0.6</td>
<td>2.6 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>69 ± 5</td>
<td>207 ± 16</td>
</tr>
<tr>
<td>Subject 6</td>
<td>4.8 ± 0.3</td>
<td>2.4 ± 0.1</td>
<td>5.4 ± 0.3</td>
<td>65 ± 3</td>
<td>270 ± 26</td>
</tr>
</tbody>
</table>

Cardiac output (CO), cardiac index (CI), circulating blood volume (BV), circulating blood volume index (BVI), and time constant of indocyanine green (ICG) metabolic disposal (TICG) for three subjects in whom the ICG fluorescence dilution curves were calibrated in units of blood ICG concentration (expressed as mean ± SD). Parameters CO and CI represent the average of six determinations with the optical probe positioned on the nose ala and on the ear lobe. Parameters BV, BVI, and TICG represent the average of two determinations from the same locations.

The area under the fluorescence dilution curve and the peak fluorescence intensity were linearly related (fig. 2) to the laser Doppler perfusion signal intensity measured within a few minutes of the fluorescence recording and from the same location (R² = 61% and 63% of variance explained, respectively). This observation suggests that local vascular density and vascular tone at the measurement site which influenced local perfusion affected in a related fashion, both the intensity of the ICG fluorescence dilution curves measured on the nose ala, the ear lobe, and the temple (mean ± SD; n = 18 [triplicate measurements of fluorescence traces on six subjects] for nose ala and ear lobe measurements; n = 9 [triplicate measurements of fluorescence traces on three subjects] for temple measurements). The mean transit time of the corrected fluorescence curve was computed as indicated in the legend of figure 1.

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cence signal and the laser Doppler perfusion measurement.

Discussion

The current study demonstrated that well-defined and reproducible fluorescence dilution curves could be measured transcutaneously in healthy humans from three sites on the skin surface of the face. The fluorescence dilution curves measured at the skin surface could be calibrated in terms of the concentration of indicator in circulating blood to calculate cardiac output and circulating blood volume values in the normal range for the subjects in the study.

The ICG fluorescence dilution traces recorded transcutaneously from the three locations on the face (nose ala, ear lobe, temple) were not different with respect to their temporal profile, although the traces differed in terms of signal intensity. The duration of the fluorescence trace is related to the temporal dynamics of the blood flow containing the fluorescent indicator in the microvascular vessels at the measurement site. The mean transit time of the transcutaneous fluorescence dilution curves was just 2–3 s longer than that obtained from radial artery blood samples after ICG injection, suggesting that perfusion was brisk and abundant at all measurement sites. The amplitude of the trace is determined in part by the amount of ICG in the volume interrogated by the measurement probe and therefore the density and depth of the subcutaneous microvessels. Our results suggest that the rate of microvascular blood flow velocity was comparable for the three measurement sites tested, probably as a result of the stable vasodilation achieved by local heating. In addition, the subjects were kept relaxed in a stable basal state, which likely reduced variations in local sympathetic vascular tone. Concurrently, the mean laser Doppler perfusion and the pulsatility of the laser Doppler signal remained constant during the exploration of each measurement site in all subjects. The blood supply of the nasal wing is more abundant and less affected by vasomotor activity than that of more peripheral locations such as the finger (Eduardo H. Rubinstein, M.D., Ph.D., Professor Emeritus, Department of Anesthesiology and Physiology, University of California, Los Angeles, California, personal verbal communication, June 2005), enabling detection of optical absorption changes associated with ICG injections by pulse dye densitometry. Reflectance oximetry studies have shown that placement of the optical sensor on the forehead above the eyebrow yields intense pulsatile signals that remain stable during peripheral vasoconstriction. Our results are similar to those of these studies and indicate that the face is a favorable site for transcutaneous ICG fluorescence dilution measurements. The nose ala and the ear lobe likely presented a larger and more vascularized tissue volume to the measurement probe, which resulted in a more intense fluorescence signal in comparison to that measured from the temple.

The reproducibility of the fluorescence dilution technique for measurement of the cardiac output estimated from the coefficient of variation of the area under the dilution curve had a median value ranging between 4% and 6% for triplicate measurements in our small group of subjects. Similar levels of reproducibility have been reported for the gold standard thermodilution technique. This coefficient of variation spanned a wider range between subjects for measurements obtained on the nose ala (approximately 1–18%) when compared with the ear lobe (approximately 2–6%), possibly because nostril flaring associated with breathing and transient contractions of the nose muscles in some subjects slightly displaced the optical probe between measurements.

Calibrated values of the cardiac output and the circulating blood volume obtained in three subjects were in the expected range. Although the number of subjects in the study was small, it is encouraging that the transcutaneous fluorescence traces recorded from two different locations on the body surface could be calibrated to produce near-identical estimates of the two parameters. This study did not examine how to efficiently calibrate the fluorescence traces in terms of circulating ICG concentration. Transcutaneous fluorescence readings were related to spectrophotometric determination of ICG content in several venous blood samples obtained after a dose of injected ICG had circulated for more than 3 min. We assumed that after that time, ICG was uniformly mixed within the circulating blood volume. Because the ICG dye is only eliminated by the liver, venous blood ICG concentration was considered to be equal to arterial ICG concentration, the traditional measurement parameter for indicator dilution techniques. Different approaches have been proposed to estimate the concentration of an exogenous optical indicator using the ratio of the fluorescent over reflected light intensities or the reflected intensities at several wavelengths. Adaptation of these methods to the current technology could eliminate the need for direct measurement of ICG content in blood samples and thereby make the fluorescence dilution method clinically practical.

The amount of ICG (1 mg) used in the subjects to produce first pass fluorescence dilution curves usable to calculate cardiac output was selected in reference to our previous laboratory studies. This amount was substantially smaller than the ICG doses (5–10 mg) commonly used in indicator dilution techniques that estimate cardiac output based on ICG absorption measurements. The fluorescence intensity returned to baseline in less than 10 min in all cases such that cardiac output procedures could be repeated every 10 min. Note that ICG elimination by the liver is not an ideal first-order pro-
The fluorescence dilution technique in healthy humans

...which explained in part that the smaller 1-mg ICG doses injected for cardiac output measurements cleared proportionally faster from the blood pool than the larger 15- to 25-mg doses used for calibration and blood volume measurements. Estimation of the circulating blood volume requires accurate measurement of the fluorescence intensity several minutes after injection at a time when the dye not yet eliminated by the liver is assumed to be homogeneously mixed in the circulating blood volume. Although smaller ICG amounts than those used in this initial study to calculate circulating blood volume would likely produce interpretable fluorescence dilution traces, blood volume assessment requires larger ICG doses than cardiac output measurements.

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References


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